

[0080] When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labeled antibody is allowed to bind to the first antibody-molecular marker complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the molecular marker of interest. Immunofluorescence and EIA techniques are both very well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

EXAMPLE 2

Production of Serum and Solutions Containing Antibodies

[0081] The following procedure or a variation can be used for any vertebrate. A goat is inoculated by intramuscular injection with cancer cells or their derivatives using an intramuscular injection. Blood samples are drawn after an appropriate interval, such as two weeks, for initial assessment. In the optimized procedure, the goat is injected every week for four weeks, then at six weeks the animal is then bled to obtain the reagent.

[0082] Approximately 400 cc of blood is drawn from the goat under sterile technique. The area for needle extraction is shaved and prepared with betadine. An 18-gage needle is used to draw approximately 400 cc of blood from the animal. Of note is that the animal can tolerate approximately 400 cc of blood drawn without the animal suffering any untoward effects. The animal does not have to be sacrificed. The animal can then be re-bled in approximately 10 to 14 days after it replenishes its blood volume.

[0083] The presence of potentially useful antibodies is confirmed. Once the presence of such reagents is confirmed blood is then taken from the goat at between 4-6 weeks, and centrifuged to separate the serum. 300 ml of serum is then filtered to remove large clots and particulate matter.

[0084] The serum is then treated with supersaturated ammonium sulfate (45% solution at room temperature) to precipitate antibodies and other material. The resulting solution is centrifuged at 5000 rpm for five minutes, after which the supernatant fluid is removed. The precipitated immunoglobulin is resuspended in phosphate-buffered saline ('PBS buffer', see Sambrook et. al. 'Molecular cloning, A Laboratory Manual', 1989) sufficient to re-dissolve the precipitate.

[0085] The solution is then dialyzed through a membrane with a molecular weight cut off of 10,000 Daltons. Dialysis is carried out in PBS buffer, changed every four hours over a period of 24 hours. Dialysis is carried out at 4° C.

[0086] After 24 hours of dialysis the contents of the dialysis bag are emptied into a sterile beaker. The solution is adjusted such that the mass per unit volume=10 mg per ml. The dilution is carried out using PBS. The resulting solution is then filtered through a 0.2 micron filter into a sterile container. After filtration, the solution is aliquoted into single doses of 1 ml and stored at -22° C. prior to use. The reagent is then ready for use.

[0087] Changes may be made in this procedure, such as for example by varying the concentration of the ammonium sulfate or switching to other reagents. Similarly, the dialysis cut-off need not be at 10,000 Daltons.

1. A method for identifying cancer-specific antibodies utilizing an immune checkpoint inhibition treatment in a subject who has cancer comprising the steps of:

- a. obtaining cancer cells and/or their components, and normal cells and/or their components from the subject who has cancer;
- b. administering the immune checkpoint inhibition treatment to the subject who has cancer;
- c. allowing time for generation of an immune reaction against the cancer cells in the subject who has cancer;
- d. obtaining a serum sample from subject who has cancer, the sample comprising any antibodies which may have been generated against the cancer cells;
- e. optionally discontinuing the immune checkpoint inhibition treatment of the subject who has cancer;
- f. removing from the serum of step d antibodies that bind to the normal cells and/or components by incubating the serum with a cell culture prepared of the normal cells and/or their components of step a, allowing sufficient time for antibodies/antigens binding and generation of antibodies/antigen complexes, and removing and keeping the supernatant;
- g. selecting the antibodies that bind to the cancer cells and/or their components by incubating the supernatant of step f with a culture of the cancer cells and/or their components of step a, allowing sufficient time for antibodies/antigens binding and generation of antibodies/antigen complexes, keeping the culture comprising antibodies/antigen complexes for further analysis and discarding the supernatant;
- h. extracting the antibody/antigen complexes from the culture of step g;
- i. separating the antibodies and the antigens of the antibody/antigen complexes of step h using standard protocols;
- j. analyzing the antigens of step i are using standard methods for antigen identification, such as mass spectrometry; and,
- k. identifying sites or regions on the antigens of step j which are specific to the cancer cells or their components obtained from the subject who has cancer in step a.

2. A method of using the sites or regions identified in step k of claim 1 to produce a medication, wherein the medication may be antibodies specific to the sites or regions, or other therapeutic agents specific to the sites or regions, or to related sites or regions.

3. A method of treating a subject who has cancer, comprising administering to said subject a therapeutically effective amount of a pharmaceutical composition comprising the medication according to claim 2.

4. A method of treating a subject who has cancer, comprising administering to said subject a therapeutically effective amount of the isolated antibodies of step i of claim 1, wherein the antibodies are either unmodified or modified.

5. The method of claim 1, wherein the structure of at least one of an antigen's antibody/antigen attachment sites of the antibodies/antigens complexes of step i is determined, and the antigen's antibody/antigen attachment site is used as a treatment target for non-antibody based cancer treatments.